Most infants first come into contact with microbes during birth — or so researchers have assumed.

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Baby's first bacteria

THE WOMB WAS THOUGHT TO BE STERILE. SOME SCIENTISTS ARGUE IT'S WHERE THE MICROBIOME BEGINS.

By Cassandra Willyard

Soon after conception, a human embryo begins to assemble a remarkable organ crucial to its survival. The placenta is both a lifeline and a guardian: it shuttles oxygen, nutrients and immune molecules from the mother's bloodstream to her developing fetus, but it also serves as a barrier against infections. For more than a century, doctors have assumed that this ephemeral structure — like the fetus and the womb itself — is sterile, unless something goes wrong.

Starting around 2011, Indira Mysorekar began questioning this idea. She and her colleagues had sliced and stained samples from nearly 200 placentas collected from women giving birth at a hospital in St Louis, Missouri. When the researchers examined the samples under a microscope, they found bacteria in nearly one-third of them[1]. "They were actually inside cells there," says Mysorekar, a microbiologist at Washington University in St Louis.

Bacteria often signal infection, and infections are a common cause of premature birth. But the microbes that Mysorekar observed didn't seem to be pathogens. She didn't see any immune cells near them; nor did she see signs of inflammation. And bacteria weren't present only in the placentas of women who gave birth early; Mysorekar also found them in samples from women who had normal, healthy pregnancies. "That was our first hint that this may be like a normal microbiome," she says.

Studies seeking to understand how microbes help to shape human health and development have become extremely popular over the past few decades, but some researchers are concerned that a crucial question — when bacteria first colonize the body — has not yet been answered. Doctors have assumed that the first contact with colonizing bacteria occurs in the birth canal. Clinicians are even looking to see whether babies born by caesarean section might benefit from a swab of their mother's vaginal microbes. But Mysorekar and other scientists have found evidence of bacteria in the placenta, amniotic fluid and meconium — the tar-like first stool that forms in a fetus *in utero*. This has led some researchers to posit that the microbiome might be seeded before birth.

If that is true and bacteria are a normal — perhaps even crucial — part of pregnancy, they could have an important role in shaping the developing immune system. Scientists might be able to find ways to shift the microbial composition in the womb and possibly ward off allergies, asthma and other conditions. They might also be able to uncover microbial profiles associated with preterm birth or other complications during pregnancy, which could help to illuminate why they occur.

The scientists at the centre of these discoveries argue that the dogma of a sterile womb is on its way out. Perhaps humans, like species such as clams, tsetse flies and turtles, can inherit a mother's microbes before they are even born[2]. "If we do not have microbes *in utero*, I think we would be the only species that has been interrogated that doesn't," says Susan Lynch, a microbiologist at the University of California, San Francisco.

"IF WE DO NOT HAVE MICROBES *IN UTERO*, I THINK WE WOULD BE THE ONLY SPECIES THAT HAS BEEN INTERROGATED THAT DOESN'T."

But even as the number of papers supporting this idea grows, some scientists are pushing back. "I just don't think that these microbiomes exist," says Jens Walter, a microbiologist at the University of Alberta in Edmonton, Canada. Where some see an intriguing new avenue of research, others see biological implausibility, sloppy science and a spectre that has long haunted microbiome research — contamination. Now, studies are getting under way that could answer the question once and for all.

One paediatrician likens the controversy over the placental microbiome to a scientific "knife fight". But if fetal microbiomes do exist, that could have far-reaching implications not only for medicine, but also for basic biology. "If we start thinking of the placenta as a conduit or facilitator of maternal-fetal communication and not as a barrier, then I think we open ourselves up to very interesting perspectives on how we've interpreted a lot of developmental biology today," says Kjersti Aagaard, an obstetrician at Baylor College of Medicine in Houston, Texas.

PROBING THE PLACENTA

The sterile-womb dogma goes back to French paediatrician Henry Tissier, who investigated the source of a baby's first bacteria around the turn of the twentieth century. Researchers began to find bits of evidence against sterility more than three decades ago, but the idea that the placenta might harbour a fully fledged microbiome didn't gain much attention until 2014, when a team of researchers led by Aagaard identified bacterial DNA in placental tissue[3].

Aagaard, who was working on the Human Microbiome Project, noticed something odd. Babies were supposed to get the bacteria that will become their microbiome in the birth canal, but she saw a mismatch between the bacteria present in the vaginas of pregnant women and those present in infants in their first week of life. That might make sense, she thought, if the microbiome gets seeded before birth.

Aagaard reasoned that if mothers were passing bacteria to their babies in the womb, there might be evidence of that transfer in the placenta, the organ that connects the two. To investigate, she and her team harvested tiny bits of tissue under sterile conditions from the placentas of 320 women, including some who gave birth early and some who had infections during pregnancy. Bacteria can be difficult to culture. So, to identify what was there, they used gene sequencing. They took biopsies of the placentas in a sterile room within an hour of delivery, sliced off the surfaces to avoid contamination, and placed those samples into vials. They also analysed the contents of empty vials to rule out contamination from the environment or the DNA-extraction reagents.

Not every placenta contained detectable bacterial DNA, but many did[3]. To get a more in-depth picture of the capabilities of these microbes, the researchers performed whole-genome sequencing on a subset of the samples. In most, they found communities dominated by *Escherichia coli* and a few other groups. And when they compared the bacterial DNA from placentas with that from bacteria typically found in other areas of the body, the results best matched the kinds of microbe found in the mouth. How oral bacteria would have made their way to the placenta isn't clear, but one possibility is that they travelled through the bloodstream. Even routine tooth brushing can allow bacteria access to the blood. What's more, the microbial signature seemed to differ in women who had experienced a preterm birth or an earlier infection. Physicians have assumed that the mere existence of bacteria in the placenta signals infection, but to Aagaard it seemed clear that which bacteria are present is much more important than whether they are there at all.

The paper made a splash in the popular press, but critics argued that Aagaard was overreaching. "DNA is not bacteria," says Mathias Hornef, head of the Institute of Medical Microbiology at the University Hospital RWTH Aachen in Germany. DNA can be used to characterize a microbiome, he says, but not to establish its existence.

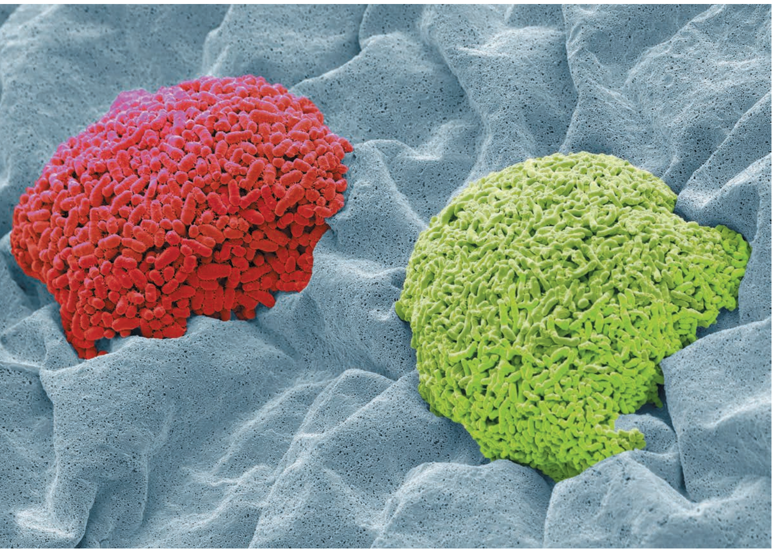
Aagaard's findings weren't an isolated event, however. Several other groups have found bacterial DNA and more in the placenta. Mysorekar, for example, saw the host of bacterial structures inside cells taken from the placenta[1]. And in 2016, a Finnish group managed to culture bacteria from placental tissues taken from women who had healthy pregnancies[4].

Researchers have also found bacteria in amniotic fluid[4,5], leading them to wonder whether the fetus might occasionally ingest microbes when it swallows some of that fluid. And some researchers, including Josef Neu, a neonatologist at the University of Florida in Gainesville, identified bacterial DNA in meconium[6], a finding that suggests the fetus's gut itself may harbour bacteria before birth. Some of the DNA came from the same genera found in amniotic fluid. And the results showed that the microbes in the stool of preterm infants were different from those in babies born at full term.

Neu hypothesized that some strains of bacteria might prompt the fetal gastrointestinal tract to produce inflammatory proteins that would trigger early labour. And indeed, some studies[7] have shown that amniotic fluid from premature babies does hold more of these proteins. That association doesn't prove anything, but it does provide "some interesting pieces of the puzzle", he says. "The fetal–maternal microbiome may be at least a partial explanation for some of these cases of preterm delivery."

Lynch's group is one of several that have been able to culture bacteria from meconium. But it's not yet clear whether those bacteria are simply passing through the fetus, or whether they're actually growing, dividing and taking up residence in the fetal gut, she says. Lynch is now looking at human fetal tissue to see whether she and her colleagues can find evidence of bacteria in the intestinal lining.

A handful of animal studies suggests that this kind of bacterial transfer from mother to fetus is possible. In the mid-2000s, a team of researchers led by microbiologist Juan Miguel Rodríguez at the Complutense University of Madrid inoculated pregnant mice with labelled bacteria, and delivered the pups by caesarean section. They found the


STEVE GSCHMEISSNER/SPL

Bacterial culture from a belly button: there is some debate as to how different parts of the body are first seeded with microbes.

labelled bacteria in both the amniotic fluid[8] and pups' meconium[9].

"What we're seeing in these animal models and what we're seeing in humans really seems to support this fetal–maternal microbiome," says Neu. "I'm not 100% convinced, but I think the data is becoming very strong."

CONTAMINATION QUESTIONS

A number of researchers, however, remain deeply sceptical. The traces of placental microbes, they argue, are 'kitome' — contaminants from the DNA-extraction kits used in the research. There's some evidence to support this. Samuel Parry, a perinatologist at the University of Pennsylvania's Perelman School of Medicine in Philadelphia, was initially intrigued by Aagaard's data. So he planned a study to examine differences between the placental microbiomes of preterm infants and those of babies born at term. As a first step, his team sought out trace amounts of DNA found on sterile swabs, reagents, DNA-purification kits and other equipment that they would routinely use. The bacterial DNA that they ultimately recovered from six placenta samples was indistinguishable from that found on the extraction kits[10]. They've since tested several dozen placentas, Parry says. "We just can't find a microbiome." Marcus de Goffau, a microbiome researcher at the Wellcome Sanger Institute in Hinxton, UK, says that he and his colleagues have similar unpublished results from "hundreds" of placentas.

One of the problems, he says, is that any bacterial signal in the placenta would be weak. In faeces or saliva, there are so many bacteria that it's easy to distinguish the microbiome from background contamination. But when microbes are scarce, a true signal is much harder to pick up. The problem goes much further than studies on human fetuses, he adds: "The entire sequencing field is littered with nonsense."

Aagaard stands by her results. "We are very cautious," she says. "Could we be misinterpreting things? Of course. But we have put in the negative and positive controls every place we can." And she points out that several other groups have found evidence of bacterial DNA in the placenta.

Parry and obstetrician Roberto Romero at the National Institute of Child Health and Human Development in Detroit, Michigan, are planning a multi-centre study to examine the question in even more placentas. They hope to hold a meeting to design the protocol in the next couple of months. If all goes well, they could have an answer as soon as next year, Romero says. They have invited Aagaard to participate, and she says she is willing. "Kjersti Aagaard is an outstanding investigator and she has put forth an idea that is interesting, is important and deserves to be tested," Romero says. "This controversy can be solved."

They aren't the only ones looking for answers. de Goffau is part of a team that has received a £1.6-million (US$2-million) grant from the UK Medical Research Council to examine placental tissue and blood for infectious agents that might be correlated with pregnancy complications. And last year, the US National Institutes of Health announced that it would offer funding for research into the early development of the immune system. The announcement specifically mentioned studies to examine how the fetal microbiome gets seeded and evolves, and how that might impact the brain.

If research fails to detect a microbiome in the womb, that doesn't eliminate the possibility that the fetus might encounter microbes there. "There's very little in and on the human body that could be considered sterile," says Juliette Madan, a neonatologist at Dartmouth–Hitchcock Medical Center in Lebanon, New Hampshire. But a handful of microbes does not necessarily mean there's a complex, thriving microbiome. Madan doesn't expect researchers to find any meaningful sharing of bacteria between mother and fetus.

But de Goffau, one of the most vehement critics of the placenta papers, isn't so sure. He has himself managed to detect bacteria in meconium. "It's not completely sterile. That's pretty clear," he says. Although the evidence isn't complete, he adds, a fetal microbiome is at least possible.

Maria Dominguez-Bello, a microbial ecologist at New York University, runs a study looking at the development of the infant microbiome and the potential benefits of putting babies in contact with their mothers' vaginal microbes after a caesarean section. She doesn't find the reports of bacteria in meconium all that convincing, however. She argues that sterility is broken when the amniotic sac breaks, which leaves plenty of time for bacteria to make their way into the infant's gut. "Labour takes hours, during which the baby is swallowing and rubbing against the walls of the birth canal," she adds. Even if a baby is born by caesarean section, it might take hours or even days for the infant to pass its first stool — a window during which it might acquire bacteria outside the womb.

The most compelling evidence that a fetal microbiome doesn't exist, say Dominguez-Bello and others, is the existence of laboratory mice that are free of bacteria. To create these germ-free rodents, pups are surgically delivered from mothers with normal microbiomes and then raised under sterile conditions. "We've done these experiments, and we've done them for 70 years," Walter says. If just one bacterium were present inside the pup, it would quickly colonize, and the protocol would fail. It would be impossible to complete such experiments.

"I would argue that if you talk with real microbiologists, they wouldn't consider it controversial," says Walter. The question, he adds, has already been answered.

Mysorekar, who is a microbiologist, disagrees. Some people are stuck on the idea that the placental microbiome is "fake news", she says. That, she argues, is a shame. "There are some very exciting questions to address." Humans start to develop a repertoire of immune cells while still in the womb, Mysorekar says, which suggests some sort of microbial exposure. She wonders where these microbes come from and how the exposure occurs. "There's so much to learn," she says. But she isn't surprised by the scepticism. In any emerging field, she says, you'll find "some naysayers, some dirty data, but also a lot of compelling new observations which together push the field forward". ■

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